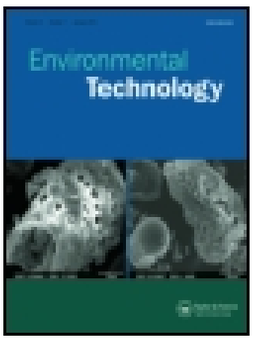

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Use of soil fungi in the biosorption of three trace metals (Cd, Cu, Pb): promising candidates for treatment technology?

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Abstract

Trace metal contamination is a widespread and complex environmental problem. Because fungi are capable of growing in adverse environments, several fungal species could have an interesting potential in remediation technologies for metal contaminated environments. This study proposes to test the ability to tolerate and biosorb three trace metals (Cd, Cu and Pb) of 28 fungal isolates collected from different soils. First, a tolerance assay in agar medium was performed. Each isolate was grown in the presence of Cd, Cu, and Pb at different concentrations. Then, we exposed each soil fungus to 50 mg L^{-1} of Cd, Cu, or Pb during 3 days in liquid medium. Parameters such as biomass production, pH, and biosorption were evaluated. The results showed that responses to metal exposure are very diverse even with fungi isolated from the same soil sample, or belonging to the same genera. Several isolates could be considered as good metal biosorbents and could be used in future mycoremediation studies. Among the 28 fungi tested, *Absidia cylindrospora* biosorbed more than 45% of Cd and Pb, *Chaetomium atrobrunneum* biosorbed more than 45% of Cd, Cu, Pb, and *Coprinellus micaceus* biosorbed 100% of Pb.

Keywords: biosorption, metal, soil fungi, tolerance, bioremediation

Count word : 6553

Introduction

Soil contamination is a widespread problem in many countries. Trace metals and polycyclic aromatic hydrocarbons (PAH) contamination of lands are some of the most important and complex types of pollution in the world [1]. Several studies already showed the bioremediation potential of fungi in PAH biodegradation [2–4]. The behavior of trace metals is hardly

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predictable because of their dynamic physico-chemical properties shaped by the properties of the soil matrix [5–7]. Cadmium (Cd), copper (Cu) and lead (Pb) are three frequent and toxic trace metals found as soil contaminants. Metals are naturally present in soils. Some of them are essential micronutrients (Cu, Co). All the metals could become toxic when they are present in bioavailable quantities that exceed toxicity thresholds for the living organisms [8]. Metals are unmetabolized or slightly metabolized [9]. Biodegradation methods using living organisms are therefore not applicable, except for volatilization techniques concerning metalloids like mercury or selenium, but such technology could result in secondary metal spreading [10–12]. Biostabilization and bioaccumulation strategies are low-cost and more appropriate. They could effectively reduce bioavailability and toxicity of the trace metals targeted. Bioextraction is another effective strategy which allows to reduce the amounts of pollutants [13]. Many organisms including bacteria [14–16], plants and the rhizosphere [17–19], as well as fungi [20–22] are able to biosorb trace metals. Several studies give information about metal remediation with fungi [7, 23–28]. Due to their efficient adaptation in mineral nutrients intake and their high tolerance to unfavorable, even toxic environments, fungi have a strong potential in trace metals bioaccumulation in order to rehabilitate soils [9, 29, 30].

The bioaccumulation of trace metals by mycelium involves biosorption interactions between the metallic ions and the cell wall, including chemical groups like -COOH, -SH, -OH, $-NH_2$, $-PO_4H_2$ [22, 31]. Several authors showed that metal biosorption by fungi generally suited the Langmuir or the Freundlich model, which is also in agreement with the central role of the fungal cell wall [32–34]. Metallic environments may induce the fungal production of secondary metabolites (oxalic acid, metallothionein, thiols) as detoxifiers which can shape trace metal mobilization and enhance interactions with the cell wall and the biosorption process [7, 9, 35–40]. Ceci et al. [41] also showed that oxalate is one of the main fungal agent of Pb biomineralization. However, Rhee et al. [42] showed that fungi could also turn Pb into an

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insoluble form (pyromorphite, $Pb_5(PO_4)_3Cl$), which becomes immobilised, consequently avoiding its biosorption and toxicity. Thus, Zhao et al. [43] identified more than 50 upregulated genes of *Lentinula edodes* after Cd exposure. Many cell functions were related to these genes, like metabolism, energy, cellular transport and communication, transcription. These studies highlight the complex interactions between fungi and trace metals.

The aim of this study is to evaluate the abilities of several fungal isolates to tolerate and biosorb Cd, Cu and Pb. A screening experiment including 28 fungal isolates collected from different soils was performed to select efficient fungi before their use in soil microcosms. Many studies focused on a short number of isolates and the numerous protocols in biosorption studies make the comparison difficult between the isolates. The 28 isolates of the present study are all tested with the same protocol, which can allow comparing them objectively and highlighting the most efficient for further studies.

Materials and methods

Fungal isolates

Table 1 gives an overview of the 28 fungal isolates and their origins. Twenty-one fungi were isolated from industrially contaminated sites following Warcup's soil plate method [44] and seven other fungi were isolated from sporophores growing on a natural forest soil. Small pieces of sporophores (4 x 4 mm) were superficially disinfected with 70% alcohol during 1 min, and plated in malt extract agar (MEA) medium until the development of mycelium. The mycelium grown was immediately subcultured on fresh MEA medium. All isolates were preserved on MEA or potato dextrose agar (PDA) in the laboratory mycological bank (stored at 4°C).

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The identification of the isolates was based on both morphological and molecular analyses. The morphological analyses was performed by visual and microscopic observations (Olympus CX 31®), looking for morphological identification characteristics [45–47]. The molecular identification was based on ITS sequencing, using ITS1/ITS4 (5'-TCC GTA GGT GAA CCT GCG G-3' - 5'-TCC TCC GCT TAT TGA TAT GC-3') or ITS5/ITS4 (5'-GGA AGT AAA AGT CGT AAC AAG G-3' - 5'TCC TCC GCT TAT TGA TAT GC-3') primers [48–50]. The DNA was first extracted from a pure culture on agar media using a specific kit (Nucleo Spin Plant® II, Macherey-Nagel®) following the protocol of the manufacturer. A PCR was performed to obtain the targeted amplicons (annealing temperature: 52°C, 30 cycles within a thermocycler Eppendorf Mastercycler Personal®). The sequenced amplicons (GATC®) were compared with databanks like BLAST (NCBI) and MycoBank [51]. An identification was considered positive when the analogy and recovery were at least equal to 98% and were confirmed by microscopic observations.

Trace Metals and analyses by ICP-OES

The following salts were used in every experiment: cadmium sulfate ($CdSO_4$), copper sulfate pentahydrate ($CuSO_4$, 5 H_2O), and lead nitrate ($Pb(NO_3)_2$) (Sigma Aldrich®, analytical grade). Stock solutions (1g L^{-1}) were prepared by dissolution of the corresponding salt mass in ultra-pure water and stored at 4°C. These stock solutions were used to prepare the metal-enriched media. Trace metals were analysed by argon Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES 5100 – Agilent technologies®) in every experiments. The analytical quality of chemical data was validated using standard certified materials HR-1 (sediments, Canadian Center for Inland Waters National Laboratory for Environmental Testing) and CSM-2 (*Agaricus campestris*, LGC Standards®). The values of 0.005, 0.03 and

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0.03 mg L^{-1} are respectively the ICP-OES limits of detection for Cd, Cu and Pb, calculated as three times the standard deviation of ten blank replicates [52].

Tolerance in agar media

Metal-enriched media (MEA or PDA) containing 10; 50; 100 mg L^{-1} of one metal were prepared by diluting the stock solution in the media; for 1000 mg L^{-1}, the appropriate mass of salt was directly dissolved in the media before solidification. Each strain was centrally deposited in enriched-MEA or PDA, in Petri dishes, and incubated at 25°C. During 30 days, the growth diameters were recorded every day and compared, for each strain, to the growth diameter obtained without metal (referred as control), in the same conditions. The tolerance assay was performed in triplicate for each isolate, each metal, and each metal concentration. The highest tolerated concentrations, which allowed visual growth of the mycelium (compared to control) were recorded for each metal.

Biosorption in liquid medium

A preliminary study using *Absidia cylindrospora* (referred as number AcS_2000 in the laboratory collection) allowed to define parameters such as exposure times and metal concentrations [53]. This previous study showed that fungal biosorption abilities are higher after an exposure of 3 days at 50 mg L^{-1} for these three metals. These conditions were also mentioned with others strains (Ascomycota and Basidiomycota) in previous studies [29, 32, 54–56].

First, a subculture of 10 or 20 days on the appropriate solid media (MEA or PDA) at 20 °C was necessary to obtain about 30 mg of fungal biomass (dry mass). Fresh mycelium was harvested from the subculture and transferred in 25 mL of the appropriate broth media (Table 1). After a period of 3 days at 20°C, on a rotary shaker at 150 rpm, 50 mg L^{-1} of Cd, Cu or Pb were added in the liquid medium. After 3 days of exposure (20°C, 150 rpm), the mycelia

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were filtered on sterile gauze, then gently rinsed twice with 25 mL of distilled water in order to desorb trace metals eventually adsorbed on the mycelium cell wall. The dried mycelia (40°C, at least 72h until constant mass) were then placed in Teflon® hermetic tank and 10 mL of *aqua regia* were added to digest the dried biomass as previously described [53]. Each fungal isolate was tested in triplicate with each metal. Trace metals were then analysed in the rinsing water and in the digests (ICP-OES 5100 – Agilent technologies®). The percentages of absorbed ($\%_{ABS}$) and adsorbed ($\%_{ADS}$) metal were then determined as the quantity of metal absorbed (Q_{ABS}), or adsorbed (Q_{ADS}), by the mycelium divided by the initial quantity of metal in the culture media (Q_0). Biosorption percentages were calculated as the sum of adsorption and absorption.

$$\%_{ABS} = Q_{ABS}/Q_0 \quad (1)$$

$$\%_{ADS} = Q_{ADS}/Q_0 \quad (2)$$

To give complementary information, the biosorption was also evaluated as the quantity of metal (µg) biosorbed per mg of dry mycelium biomass (C_{BIOS}). A control experiment, without metal, was performed in triplicate for each fungal isolate.

The pH of the media was measured before and after metal exposure. The fungal biomass produced at the end of the experiment was also recorded. The pH and the biomass of each fungal isolate were compared to their relative controls. The pH variation (ΔpH) is given as the variation between the control (pH_C) and the experiment (pH_E):

$$\Delta pH = pH_E - pH_C \quad (3)$$

The biomass production is given as a percentage of the controls (growth without metal).

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Statistical analyses

Exploratory analyses were conducted to highlight correlations between different parameters such as biomass, biosorption percentages, pH, and finally the metal concentration inside the mycelium, by using the correlation exact test. Statistical significance was defined as $P<0.05$. All analyses were conducted using the SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Cadmium

Absidia cylindrospora is the only isolate able to tolerate Cd until 1000 mg L^{-1} (Table 2). Eleven of the twelve Ascomycota tested in the study tolerated up to 100 mg L^{-1} (Table3), five of them belong to the Sordariales order. Only half of the Basidiomycota (6/15) tolerated up to 100 mg L^{1} of Cd (five Agaricales and one Polyporales).

The mean of the biomass production after Cd exposure reached 68% of the controls (from 36% to 100%). The mean of the pH variations after Cd exposure reached +0.2 compared to the controls (from -0.5 with *Humicola sp* to +1.8 with *Pleurotus ostreatus*). These variations in pH were not statistically different ($p = 0.06$) from the initial pH of the culture medium.

The minimum biosorption was of about 10% and eight species biosorbed more than 30% of Cd and up to 14.2 mg per g of dry mycelium (Figures 1 and 2). The statistical analysis showed that the Cd biosorption percentages were respectively correlated with the concentrations of Cd inside the mycelium ($r = 0.30179$; $p = 0.0053$). The maximum was reached by *Emericellopsis minima* (49%), followed by *Chaetomium atrobrunneum* (51%), *Absidia cylindrospora* (43%), *Perenniporia fraxinea* (41%), *Coprinellus xanthothrix (41%), Agaricus arvensis* (36%), *Sordaria lappae* (34%), and *Humicola sp* (33%). Six of these species were

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isolated from metal contaminated soils (soils 3, and 4), but the Cd concentrations were inferior to our detection limit. *A. cylindrospora* has been isolated from a soil whose Cd concentration was evaluated at 12 mg kg^{-1} (soil 1). *Agaricus arvensis* came from a natural forest soil (soil 2) in which Cd was quantified at 1.5 mg kg^{-1}. During the Cd exposure experiment, *Humicola sp* and *H. fuscoatra* revealed different behavior despite their taxonomic proximity. Even if they both tolerated up to 100 mg L^{-1} of Cd in agar medium, *Humicola sp* biosorbed much more Cd (33%) than *H. fuscoatra* (7%). These two species were isolated from metal contaminated soils without Cd (soils 3 and 4). The two *Chaetomium* species of the study were isolated from the same soil with no Cd contamination (soil 3) but tolerated Cd up to 100 mg L^{-1}. However, *Chaetomium atrobrunneum* biosorbed 51% of Cd whereas *C. gracile* only biosorbed 29%. The two *Coprinellus* isolates show similar Cd tolerance (100 mg L^{-1}) but different biosorption abilities (40 and 27%). The two *Pleurotus* (28 and 23%), and the two *Agaricus* (35 and 23%) isolates also show similar Cd tolerance and biosorption abilities. Ascomycota and Basidiomycota show similar results in Cd biosorption percentages, respectively 23.8 and 21.5% (Table 4).

Copper

Seven Ascomycota and five Basidiomycota tolerated Cu up to 100 mg L^{-1}. *Fusarium oxysporum* is the only isolate able to tolerate Cu up to 1000 mg L^{-1} in agar medium (Tables 2 and 3).

The mean of the biomass production after Cu exposure reached 72% of the controls (from 33% to 100%). The mean of the pH variations after Cu exposure reached -0.4 compared to the controls. These variations in pH are statistically different ($p = 1.69.10^{-9}$) from the initial pH of the culture medium. We observed a decrease in pH after Cu exposure except for *Pleurotus pulmonarius* (+1.1) and *Coprinellus micaceus* (+1.1).

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Biosorption rates reached a maximum of 42% with *Chaetomium atrobrunneum* and 13.2 mg per g of dry mycelium for *Phaeophlebiopsis peniophoroides* (Figures 1 and 2). Only five other species biosorbed more than 30% of Cu: *Sordaria lappae* (38%), *Perenniporia fraxinea* (35%)*, Pseudeurotium bakeri* (33%), *Emericellopsis minima* (33%) and *Humicola sp* (32%). These isolates (except *Pseudeurotium bakeri)* were also among the most efficient in Cd biosorption (Figure 1). They were isolated from a soil contaminated by Cu (345 mg kg^{-1} in soil 3, and 100 mg kg^{-1} in soil 4). According to our results, fungi isolated from Cu-contaminated soil were the most tolerant and the best biosorbents for this metal, except for the two *Agaricus* species.

As for Cd, biosorption of Cu by *Chaetomium atrobrunneum* (42%) was higher than biosorption by *Chaetomium gracile* (29%). However, unlike for Cd, *Humicola sp* biosorbed Cu (32%) better than *Humicola fuscoatra* (20%). The two *Coprinellus* species showed similar tolerance (100 mg L^{-1}) and biosorption percentages: 25% and 28%. The two *Pleurotus* species (24 and 28%) and the two *Agaricus* (18 and 21%) revealed similar tolerance and biosorption abilities for this metal. Despite his high tolerance to Cu and biomass production (82%), *Fusarium oxysporum* did not reveal a higher biosorption percentage than 18.2%.

Percentage of Cu biosorption by *A. cylindrospora* was lower (6.2) than the biosorption percentages observed with Ascomycota (24.5) and Basidiomycota (21.5). However, as previously described with Cd, the results of Cu biosorption by Ascomycota and Basidiomycota were similar (Table 4).

Lead

Pb is the best tolerated and biosorbed metal in the present study. Nine isolates (three Ascomycota and six Basidiomycota) tolerate up to 1000 mg L^{-1} of Pb and fourteen isolates (six Ascomycota and eight Basidiomycota) tolerate up to 100 mg L^{-1} (Tables 2 and 3).

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The mean of the biomass production after Pb exposure reached 92% of the controls (from 43% to 130%). The mean of the pH variations after Pb exposure reached +0.1 compared to the controls (from -0.4 to +1.1). These variations in pH are not statistically different ($p = 0.08$) from the initial pH of the culture medium.

Coprinellus micaceus highlighted the best biosorption percentage: after 3 days of exposure at 50 mg L^{-1}, this isolate was able to biosorb 100% of the Pb present in the medium, and 28.0 mg of Pb per g of dry mycelium (Figures 1 and 2). *Coprinellus micaceus* is also able to tolerate 100 mg L^{-1} in agar medium. The statistical analysis showed, that the Pb biosorption percentages of all isolates were respectively correlated with the concentrations of Pb inside the respective mycelium ($r = 0.58905$; $p < 0.0001$). Twenty-two isolates were able to biosorb more than 30% of Pb and eleven of them biosorbed between 70% and 90%: *Xylaria sp* (90%), *Penicillium oxalicum* (89%), *Humicola sp* (79%), *Humicola fuscoatra* (79%), *Chaetomium gracile* (78%), *Laetiporus sulphureus* (77%), *Fomes fomentarius* (76%), *Psathyrella candolleana* (74%), *Schizophyllum commune* (73%), *Phaeophlebiopsis peniophoroides* (72%), and *Megacollybia platyphylla* (72%). Among these isolates, *Megacollybia platyphylla, Laetiporus sulphureus*, and *Fomes fomentarius* came from a forest soil with a low Pb concentration (3.9 mg kg^{-1}). However, many Basidiomycota are known to biosorb toxic substances, and particularly metals like Pb, from their environment [57].

The two *Chaetomium* isolates biosorbed 78% and 68% respectively for *Chaetomium gracile* and *C. atrobrunneum.* The last biosorbed less Pb, but tolerated until 100 mg L^{-1} and biosorbed more Cd and Cu. *Humicola sp* and *H. fuscoatra* biosorbed both 80% of Pb. *Coprinellus xanthothrix* biosorbed only 21%, whereas *C. micaceus* biosorbed 100% of Pb. *Pleurotus ostreatus* biosorbed 3 times more than *P. pulmonarius* (65% versus 21%) and they both tolerated a maximum of 10 mg L^{-1} of Pb. In contrast, the two *Agaricus* revealed similarities in Pb biosorption, 33% for *Agaricus bisporus* and 39% for *A. arvensis*, and they both tolerated

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up to 1000 mg L^{-1} of Pb. Table 4 showed that Basidiomycota were more efficient to concentrate Pb (18.3 µg.mg^{-1}) than Ascomycota (9.5 µg.mg^{-1}).

Discussion

Influence of trace metal

Our results showed that Pb is the most tolerated and biosorbed metal, followed by Cd and Cu. Chen et al. [58] studied the excretion profile of *Phanerochaete chrysosporium* under Cd stress and highlighted an increase in oxalate production induced by Cd stress, confirming the detoxifying role of the oxalate. Miersch et al. (2001) revealed that thiol production by *Mucor racemosus* (Mucoromycota) was increased after Cd and Cu exposure and Jaeckel et al. (2005) showed that Cd stress induced metallothionein and phytochelatin production by fungi [59, 60]. Sazanova et al. [61] have studied the biosorption of Cu with *Aspergillus niger* and *Penicillium citrinum*. They found that Cu do not induce oxalate production by these isolates, which seems to be different than Cd and could explain some differences observed between the metals in tolerance and biosorption and also suggests that there are different ways of detoxification. For example, the decrease in pH observed after Cu exposition could suggest that some species effectively produce metabolites like organic acids. The production, or not, of such metabolites is considered as a major metal detoxification strategy. The diversity of the defense mechanisms may explain the diversity of the biosorption abilities [20, 62, 63].

Cu is the less tolerated and biosorbed metal. Cu is a well-known and widely used antifungal agent for many years. Vincent et al. [64] published a review on Cu antimicrobial properties. They mention a "contact killing" property of Cu, which can partially explain the low tolerance of some isolates in our study. According to this review, copper ions released by the culture medium cause cell alterations (more than DNA damages) and lead to cell death. The oxidative

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stress is also mentioned as an important consequence of Cu exposure, even amplified by the previous membrane damages. Membrane damages could occur here during Cu exposure, leading to cell death, and disrupting the biosorption process. Acclimatization of fungi under metal stress could partially preserve a Cu-tolerance and explain why some isolates collected from Cu-contaminated soils were more efficient in Cu biosorption.

The correlation between the biosorption percentages of Pb and the concentration of Pb inside the mycelium could illustrate the fact that absorption is largely predominant for this metal.

Influence of soil origin

Several fungal isolates were able to tolerate and biosorb trace metal even if they came from a non-contaminated soil (like *Humicola* and *Chaetomium* with Cd or *Megacollybia platyphylla* with Pb). These results suggested that fungal species could tolerate and biosorb a toxic substance without a previous contact, as an intrinsic tolerance. It is also possible that another toxic substance or stress could have enhanced a nonspecific tolerance to Cd or Pb [65]. However, fungal isolates, which came from a Cu-contaminated soil, could reveal a better biosorption percentage than isolates from a non-Cu-contaminated soil. Zotti et al. [29] collected several fungal isolates from a Cu-contaminated area and tested their potential in Cu biosorption. The strains *Trichoderma harzanium* and *Clonostachys rosea* were able to biosorb up to 20 mg g^{1}, which seems higher than the 10 mg g^{-1} biosorbed in the present work by *Coprinellus micaceus, Chaetomium gracile, Ganoderma resinaceum,* and *Petriella sp.* These isolates came from a Cu-contaminated land (soils 3 and 4).

Six isolates collected from non-Cu-contaminated areas also showed comparable results in Cu accumulation: *Pleurotus ostreatus, Agaricus bisporus, Fomes fomentarius, Psathyrella candolleana, Xylaria sp,* and *Phaeophlebiopsis peniophoroides* that came from a soil contaminated by another pollutant (PCB). Despite the comparable Cu concentrations inside

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their mycelium, the biosorption percentages of isolates originating from Cu-contaminated soils were higher.

An acclimatization of the fungal isolates to a particular toxic metal (like Cu) could enhance the biosorption abilities, as shown by Zhang et al. and Li et al. [30, 66].

Multi-metal contamination of soil may also affect the tolerance and the biosorption of specific metal species by fungal isolates. For example, high levels of Zn may allow some fungi to tolerate higher level of Cd (by increasing the biomass production) [67].

Influence of fungal taxonomy

Our results suggested that species belonging to the same genera could reveal different biosorption abilities like *Humicola sp* and *H. fuscoatra* with Cd and Cu, *Chaetomium gracile* and *C. atrobrunneum* with Cu, *Coprinellus xanthothrix* and *C. micaceus* with Pb. No link was found between the taxonomy of fungal isolates and their biosorption abilities, which was in agreement with a previous study [68]. Nevertheless, different species in a same genus could reveal similar biosorption abilities. This was observed with the two *Coprinellus* in presence of Cd and Cu, and the two *Humicola* exposed to Pb. Our results also suggested that Basidiomycetes may biosorb more Pb than Ascomycetes. The concentration inside the mycelium was 18.3 µg mg^{-1} for the Basidiomycetes and 9.5 µg mg^{-1} for the Ascomycota although similar biomass production and biosorption percentages were observed. The fungal biodiversity should be more explored with this in mind.

The performances in Cd biosorption obtained by Li et al [55] were lower than these obtained in the present work, even for the strain *Pleurotus ostreatus* they acclimatized in Cd-enriched medium. Despite the improvement of the Cd intake and the removal rate, this acclimatized strain was not able to biosorb more Cd than this used by Favero [69] or by our native forest isolate of *Pleurotus ostreatus*. These results highlighted the differences in biosorption abilities

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between isolates belonging to a same species and demonstrated the interest to compare fungal strains in a same screening protocol.

Influence of fungal biomass

As we observed with *Absidia cylindrospora*, the growth capacity and biomass production constituted an important advantage to develop a bioremediation method. Indeed a great surface of mycelium could enhance sorption interactions between metals and chemical groups of the fungal cell wall, or fungal metabolites.

Several studies already showed the biosorption potential of *Pleurotus ostreatus.* Favero et al. [69] revealed that this species was able to tolerate up to 150 mg L^{-1} and biosorb about 20 mg g^{-1} of Cd. These better performances, in comparison to the present work (20 mg L^{-1} and 5.3 mg g^{-1}) might be related to the longer duration of preculture. These conditions allowed a higher biomass production, which could enhance tolerance and biosorption processes. Vimala and Das (2011) showed that the biosorption of Cd by *Pleurotus platypus* involved membrane ion exchange, which was relevant with the significance of biomass [70].

Ganoderma lucidum (Basidiomycota) was studied by Matute et al. [71]. During the study, the authors noticed that a low Cu supplement in the growth media (50 to 100 mg kg^{-1}) could enhance biomass production. This particularity could be used in order to enhance biomass production by fungi.

Li et al. [33] revealed the biosorption of Cu by dead fungal biomass (inactivated by formaldehyde) of *Bjerkandera sp* (12.1 mg g^{-1})*, Cladosporium cladosporioides* (7.7 mg g^{-1})*, Gliomastix murorum* (9.0 mg g^{-1}) but no information was given about the percentage of Cu-removal by these strains. Nevertheless, the fact that dead fungal biomass could also be efficient in biosorption should be taken under consideration for further studies.

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It appeared that nitrate could enhance oxalate and biomass production by fungi [40,72]. Even if the use of $Pb(NO_3)_2$ in our experiment does not reduce significantly the pH, this particularity could partially explain why Pb is better biosorbed than Cd and Cu, at least by an increase in biomass production.

Selection of efficient isolates

Several isolates of the present study revealed promising bioremediation potential. Among the 28 fungi tested, *Absidia cylindrospora* biosorbed more than 45% of Cd and Pb, *Chaetomium atrobrunneum* biosorbed more than 45% of Cd, Cu, Pb, and *Coprinellus micaceus* biosorbed 100% of Pb. The strain *A. cylindrospora* has been previously described for its ability to degrade fluorene [73] and could be an interesting strain to treat multi-contaminated environments. *Irpex lacteus, Pleurotus ostreatus,* and *P. cornucopiae* are others species that could be efficient in the bioremediation of multi-contaminated environments [55,74–76].

Conclusions

This study presents the tolerance and biosorption abilities of several fungi isolated from industrial contaminated soils and natural forest soils. These fungal isolates revealed different tolerance and biosorption profiles. No relationship has been highlighted between the specific profiles and the taxonomic groups. Some isolates seemed metal specific, as *Coprinellus micaceus*, which can biosorb 100% of Pb. Some others could significantly biosorb more than one metal, for example, *Absidia cylindrospora* is able to biosorb Cd (45%) and Pb (65%), *Perenniporia fraxinea* was able to biosorb Cd (41%), Cu (35%), and Pb (46%) and *Chaetomium atrobrunneum* was able to biosorb more than 42% of Cd, Cu, and Pb. These strains could be used in further studies in order to improve their biomass production and biosorption levels. As mentioned in previous publications [77–79], the use of a consortium of microorganisms for

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their multi-metal biosorption abilities should be further investigated. In this way, fungi could be good candidates to address complex pollution.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1. Description of the soil fungal isolates

Soil origin of the fungal isolates	Division Order[a]	Family[a]	Species	Culture Medium [b]	Subculture duration (days)
Soil 1: Industrial	Mucoromycota	*Cunninghamellaceae*	***Absidia cylindrospora***	MEA	**10**
Soil 2: Forest Cd = 1.4 mg kg^{-1} Cu = 7 mg kg^{-1} Pb = 3.9 mg kg^{-1}	Basidiomycota Agaricales	*Agaricaceae*	***Agaricus arvensis***	PDA	**20**
		Agaricaceae	***Agaricus bisporus***	PDA	**20**
		Marasmiaceae	***Megacollybia platyphylla***	MEA	**20**
		Pleurotaceae	***Pleurotus ostreatus***	MEA	**10**
		Pleurotaceae	***Pleurotus pulmonarius***	MEA	**10**
	Basidiomycota Polyporales	*Polyporaceae*	***Fomes fomentarius***	MEA	**10**
		Fomitopsidaceae	***Laetiporus sulphureus***	MEA	**10**
Soil 3: Industrial Cd < DL[c] Cu 345 mg kg^{-1} Pb 160 mg kg^{-1}	Basidiomycota	*Psathyrellaceae*	***Coprinellus xanthothrix***	MEA	**10**
	Ascomycota Sordariales	*Chaetomiaceae*	***Chaetomium atrobrunneum***	MEA	**20**
		Chaetomiaceae	***Chaetomium gracile***	MEA	**20**
		Chaetomiaceae	***Humicola fuscoatra***	MEA	**20**
		Sordariaceae	***Sordaria lappae***	MEA	**10**
Soil 4: Industrial Cd < DL[c] Cu 100 mg kg^{-1} Pb 140 mg kg^{-1}	Basidiomycota	*Psathyrellaceae*	***Coprinellus micaceus***	MEA	**10**
	Ascomycota	*Trichocomaceae*	***Penicillium oxalicum***	MEA	**20**
	Ascomycota	Not assigned	***Emericellopsis minima***	PDA	**20**
	Ascomycota	*Microascaceae*	***Petriella sp***	MEA	**20**
	Basidiomycota Polyporales	*Ganodermataceae*	***Ganoderma resinaceum***	MEA	**10**
		Polyporaceae	***Perenniporia fraxinea***	PDA	**20**
	Ascomycota	*Chaetomiaceae*	***Humicola sp***	MEA	**10**
	Ascomycota	*Pseudeurotiaceae*	***Pseudeurotium bakeri***	MEA	**20**
Soil 5: Industrial PCB > 8000 mg kg^{-1}	Basidiomycota Agaricales	*Psathyrellaceae*	***Psathyrella candolleana***	MEA	**10**
		Schizophyllaceae	***Schizophyllum commune***	MEA	**10**
	Basidiomycota Polyporales	*Phanerochaetaceae*	***Irpex lacteus***	MEA	**10**
		Not assigned	***Phaeophlebiopsis peniophoroides***	MEA	**10**
	Ascomycota	*Xylariaceae*	***Xylaria sp***	MEA	**20**
Soil 6: Industrial Cu 2 mM	Ascomycota	*Coniochaetaceae*	***Coniochaeta hoffmannii***	MEA	**20**
	Ascomycota	*Nectriaceae*	***Fusarium oxysporum***	PDA	**10**

[a] Source: Mycobank

[b] MEA: Malt Extract Agar; PDA: Potato Dextrose Agar

[c] DL: Detection limit (0.0005 mg L^{-1} for Cd, 0.03 mg L^{-1} for Cu, and 0.03 mg L^{-1} for Pb)

Table 2. Tolerance, pH variations and biomass production after exposure at 50 mg L^{-1} of metal during 3 days.

Soils	Isolates	Cd			Cu			Pb		
		HTC[a]	ΔpH[b]	BP[c]	HTC	ΔpH	BP	HTC	ΔpH	BP
1	***Absidia cylindrospora***	**1000**	+0.2	92	50	+0.1	91	10	+0.4	90
2	***Agaricus arvensis***	100	-0.2	66	100	-0.4	67	**1000**	-0.2	74
	Agaricus bisporus	100	-0.1	75	100	-0.9	59	**1000**	-0.3	75
	Fomes fomentarius	50	+0.6	64	50	-0.4	61	100	+0.7	70
	Laetiporus sulphureus	10	+0.3	98	100	-0.1	99	**1000**	-0.1	111
	Megacollybia platyphylla	0	+0.1	64	10	-0.3	103	100	+0.1	73
	Pleurotus ostreatus	10	0	72	10	**-1.2**	57	10	-0.3	82
	Pleurotus pulmonarius	10	**+1.8**	84	50	**+1.1**	90	10	+0.8	105
3	***Chaetomium atrobrunneum***	100	**+1**	61	10	0	79	100	-0.3	98
	Chaetomium gracile	100	+0.3	74	50	-0.2	61	10	-0.2	93
	Coprinellus xanthothrix	100	-0.1	57	10	**-1.1**	49	100	+0.2	70
	Humicola fuscoatra	100	+0.3	64	100	-0.4	76	**1000**	**+1.1**	126
	Sordaria lappae	100	-0.2	47	10	-0.7	57	100	0	95
4	***Coprinellus micaceus***	100	**+1.4**	68	10	**+1.1**	90	100	+0.8	105
	Emericellopsis minima	100	-0.1	80	100	**-1.1**	64	100	0.1	102
	Ganoderma resinaceum	100	**+1.1**	40	50	+0.1	78	100	-0.1	70
	Humicola sp	100	-0.5	47	100	-0.8	43	100	+0.1	99
	Penicillium oxalicum	50	-0.1	64	100	-0.2	91	**1000**	0	87
	Perenniporia fraxinea	10	0	70	100	0	63	**1000**	0	87
	Petriella sp	100	+0.1	93	10	-0.6	106	100	+0.4	116
	Pseudeurotium bakeri	100	+0.3	67	100	-0.8	52	100	+0.3	93
5	***Irpex lacteus***	50	-0.1	62	10	-0.1	77	**1000**	0	120
	Phaeophlebiopsis peniophoroides	50	-0.3	75	100	-0.4	70	100	-0.1	66

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	Psathyrella candolleana	100	+0.2	94	10	-0.4	65	100	+0.2	114
	Schizophyllum commune	50	-0.3	54	50	-0.4	52	100	-0.1	100
	Xylaria sp	100	+0.3	36	100	-0.4	33	50	0	92
6	***Coniochaeta hoffmannii***	100	-0.3	88	100	**-1.2**	89	**1000**	-0.3	130
	Fusarium oxysporum	100	-0.4	49	**1000**	-0.7	82	**1000**	-0	43

[a]HTC (mg L^{-1}): Highest Tolerated Concentration of metal that allowed fungal growth on solid single metal–enriched media (MEA or PDA)

[b]ΔpH: variation of pH after metal exposure (50 mg L^{-1} 3 days) in liquid broth media

[c]BP: Biomass Production as a percentage of the control in the same conditions after metal exposure in liquid broth media (50 mg L^{-1} 3 days)

Table 3. Metal tolerance according taxonomic groups.

Metal	Highest tolerated dose ($mg.L^{-1}$)	Mucoromycota	Ascomycota	Basidiomycota
Cd	10	-	-	4
	50	-	1	4
	100	-	11	6
	1000	1	-	-
Cu	10	-	3	6
	50	1	1	4
	100	-	7	5
	1000	-	1	-
Pb	10	1	1	2
	50	-	1	-
	100	-	6	8
	1000	-	4	5

Table 4. Metal biosorption according taxonomic groups.

Metal	Biosorption ability		Mucoromycota	Ascomycota	Basidiomycota
Cd	$\%_{BIOS}$	**moy**	**43.2**	**23.8**	**21.5**
		SD	4.8	11.3	7.5
	C_{BIOS}	**moy**	**5.9**	**6.3**	**7.2**
		SD	0.6	2.6	3.3
Cu	$\%_{BIOS}$	**moy**	**6.2**	**24.5**	**20.9**
		SD	0.4	8.6	4.8

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	C_{BIOS}	**moy**	**0.9**	**6.7**	**7.4**
		SD	0.1	2.1	2.2
Pb	$\%_{BIOS}$	**moy**	**65.6**	**55.0**	**56.7**
		SD	2.2	25.4	21.4
	C_{BIOS}	**moy**	**9.3**	**9.5**	**18.3**
		SD	0.4	4.6	12.5

$\%_{BIOS}$: percentage of biosorption

C_{BIOS}: concentration of biosorbed metal ($\mu g.mg^{-1}$)

SD: Standard Deviation

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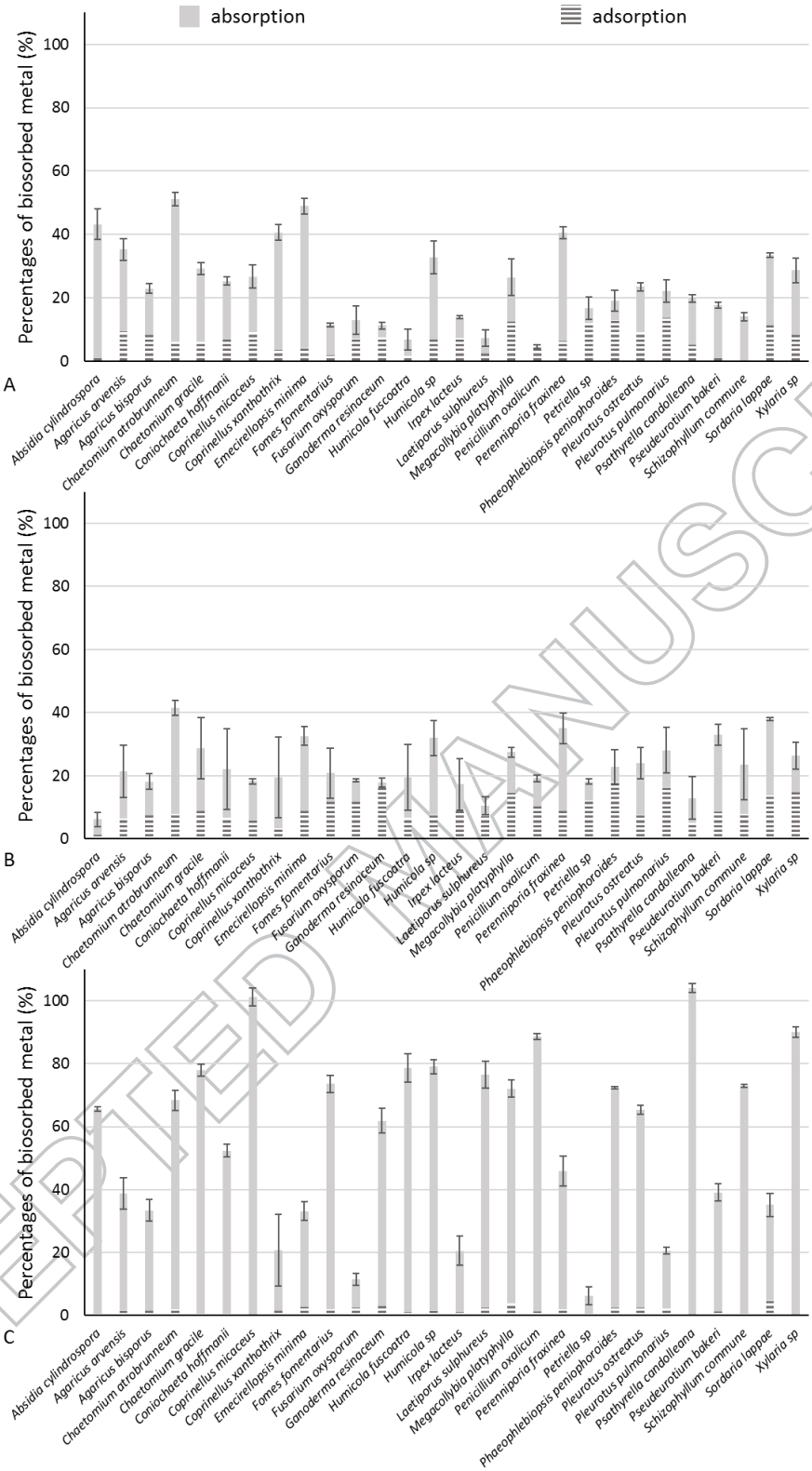

Figure 1.

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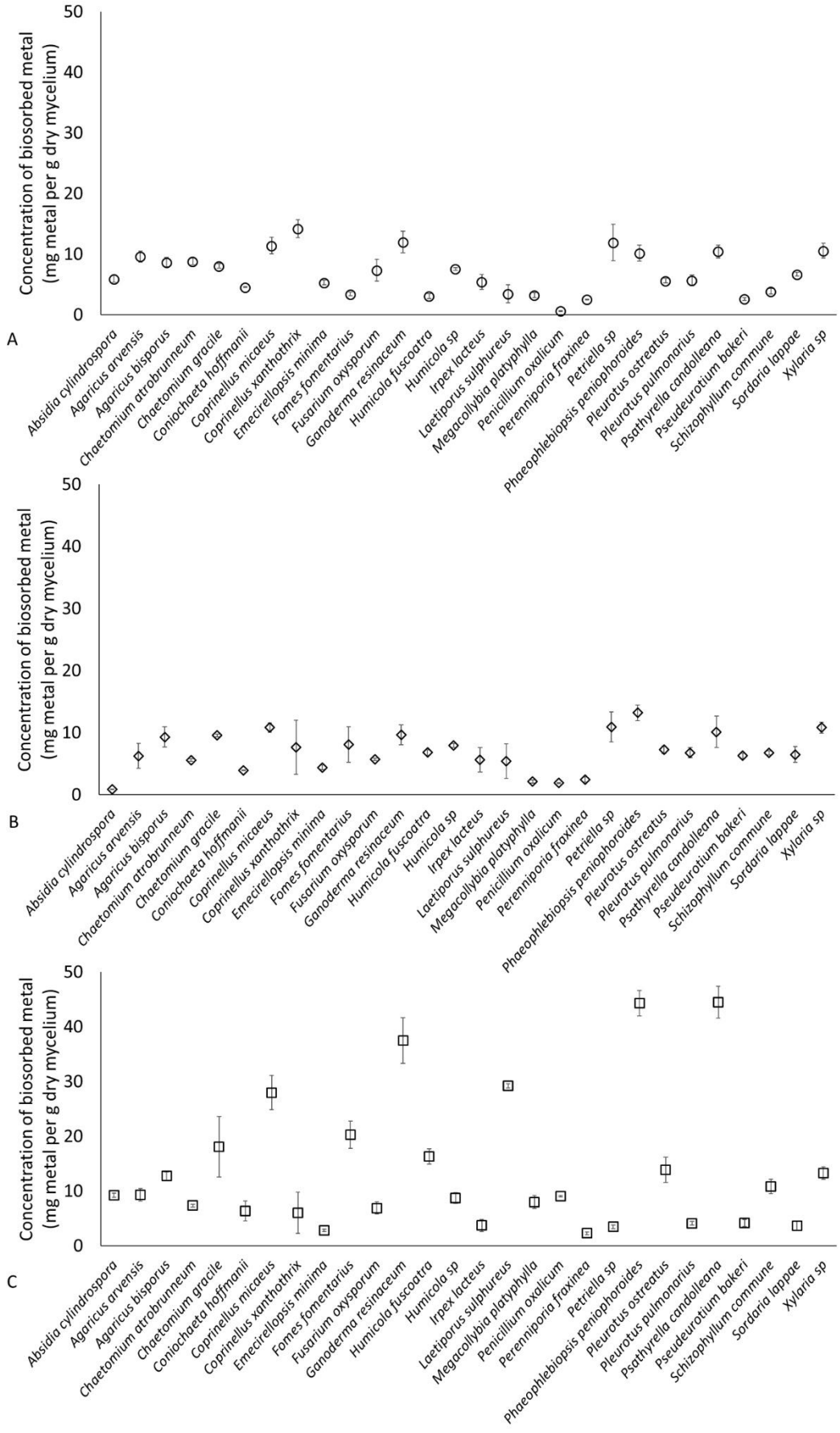
Concentration of biosorbed metal (mg metal per g dry mycelium)
50
40
30
20
10
0
A
B
C
Absidia cylindrospora
Agaricus arvensis
Agaricus bisporus
Chaetomium atrobrunneum
Chaetomium gracile
Coniochaeta hoffmanii
Coprinellus micaeus
Coprinellus xanthothrix
Emecirellopsis minima
Fomes fomentarius
Fusarium oxysporum
Ganoderma resinaceum
Humicola fuscoatra
Humicola sp
Irpex lacteus
Laetiporus sulphureus
Megacollybia platyphylla
Penicillium oxalicum
Perenniporia fraxinea
Petriella sp
Phaeophlebiopsis peniophoroides
Pleurotus ostreatus
Pleurotus pulmonarius
Psathyrella candolleana
Pseudeurotium bakeri
Schizophyllum commune
Sordaria lappae
Xylaria sp

Figure 2.

Figure 1. Percentages of adsorbed and absorbed metals after 3 days of exposure at 50 mg L^{-1} of Cd (A), Cu (B), and Pb (C). The standard deviation is mentioned for the total percentage of biosorption which is the sum of adsorbed and absorbed metal.

Figure 2. Concentrations of biosorbed metals after 3 days of exposure at 50 mg L^{-1} of Cd (A), Cu (B), and Pb (C).

ACCEPTED MANUSCRIPT